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ON THE EVOLUTION OF BIOCHEMICAL SYNTHESES

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Although it has been recognized for a long time that the biochemistry of the organism is conditioned by its genetic constitution, a more precise definition of this dependence has not been possible until recently. A considerable amount of evidence now exists for the view that there is a one-to-one correspondence between genes and biochemical reactions. This concept, foreshadowed in the work of Garrod[1] on human alcaptonuria, accounts in a satisfactory way for the inheritance of pigment formation in guinea pigs,[2] insects[3] and flowers,[4] and the synthesis of essential growth factors in *Neurospora*.[5] It appears from these studies that each synthesis is controlled by a set of non-allelic genes, each gene governing a different step in the synthesis. As to the nature of this control, it is probable that the primary action of the gene is concerned with enzyme production. That genes can direct the specificities of proteins has been shown in the case of many antigens,[6] while several mutations demonstrably affecting the production of enzymes have been reported.[6] Evidence on the postulated gene-enzyme relationship is in most cases, however, still circumstantial; this is partly because of technical difficulties involved in the study of synthetic, or free-energy consuming reactions *in vitro*, and partly because of the insufficiency of biochemical information on those reactions which happen to be susceptible of genetic analysis.

As a corollary of the above hypothesis, each biosynthesis depends on the direct participation of a number of genes equal to the number of different, enzymatically catalyzed steps in the reaction chain. In attempting to account for the evolutionary development of such a reaction chain one meets in a clear form the problem of explaining macroevolutionary changes in terms of microevolutionary steps. The individual reactions making up the chain are of value to the organism only when considered collectively and in view of the ultimate product. Regarded individually, intermediate substances cannot, in general, be assumed to have physio-

logical significance, and the ability to produce them does not of itself confer a selective advantage. An example from *Neurospora* genetics will serve to illustrate this point. At the present time seven different genes are known to be concerned in the synthesis of arginine by the mold.[7] The inactivation of any one prevents the synthesis from taking place. On the basis of the above hypothesis, at least seven different catalyzed steps must occur in the synthesis. Several of the steps have been identified and controlling genes assigned to each. Two of the intermediates in the chain have been shown to be the amino acids ornithine and citrulline. Unlike arginine, neither of these substances is a general constituent of proteins. Aside from their function as precursors, they are apparently of no further use to the organism.

While the above example probably represents the general case, there are also well-known instances in which precursors serve independent functions. Thus, arginine, glycine and methionine are precursors of creatine in the rat,[8] but the synthesis goes through the non-functional intermediate, glycocyamine. On the other hand, acetylcholine may be synthesized from choline in one step.[9] In cases such as these, the problem is that of accounting for the synthesis of the precursors.

Since natural selection cannot preserve non-functional characters, the most obvious implication of the facts would seem to be that a stepwise evolution of biosyntheses, by the selection of a single gene mutation at a time, is impossible. It will be shown below that this is not a necessary conclusion, but that under special conditions the stepwise evolution of long-chain syntheses may occur. First, however, an alternative to stepwise evolution will be considered; that is, the origin of a new reaction chain through the chance combination of the necessary genes.

Although the probability of the origin of a useful character through the chance association of many genes may be small, it is never zero. Indeed, a consideration of the statistical consequences of the interaction of mutation, Mendelian inheritance, and natural selection has led Wright[10] to the conclusion that such chance associations may be of major importance in evolution. He has analyzed the evolutionary possibilities of various types of breeding structures and has shown that under certain conditions an extensive trial and error mechanism exists, whereby the species can test numerous combinations of non-adaptive genes. The breeding structure which most favors this type of evolution is that of a large population divided into many small, partially isolated groups. Within each group the cumulative effects of the accidents of sampling among the gametes are of major significance in determining gene frequencies, but the penalty of fixation of deleterious genes, ordinarily incurred under inbreeding, is avoided by exchange of migrants with other groups. The pressures of forward and reverse mutations, which between them determine an equilib-

rium frequency for non-adaptive genes in large, random-breeding populations, become of minor importance. As a consequence, a random drift of gene frequencies occurs. If, by chance, one group finds a particularly favorable combination of genes, a process of intergroup selection comes into play, whereby the favorable combination is spread to the population at large.

This model provides a means for the evolution of a new gene combination in spite of unfavorable mutation rates to active alleles and in the absence of selection of individual genes. It is thus favorable for the evolution of systems of individually non-adaptive, but collectively adaptive, genes. The effectiveness of the process would seem to be strongly dependent on the size of the gene combination required, however, decreasing approximately exponentially with increasing numbers of genes, other factors remaining constant. There would result a tendency toward the evolution of short reaction chains involving the recombination of molecular units already available. There is no doubt that a conservative tendency of this sort actually exists in nature. The wide variety of biologically important compounds built up on the pyrrole nucleus, to mention but one example, is a case in point.

The application of Wright's theory to the particular problem under consideration is limited by the fact that it operates only under biparental reproduction. It is probable that a large number of basic syntheses evolved prior to sexual reproduction. The universal distribution among living forms of certain classes of compounds—viz., the amino acids, nucleotides and probably the B vitamins—identifies them as essential ingredients of living matter. The synthesis of these substances must have evolved very early in geologic time, as a necessary condition for further progress, although loss of certain syntheses may have occurred in the later differentiation of some forms. It is therefore desirable to search for another solution of the problem applicable to compounds of this type, preferably one in which a minimum burden is placed on chance and a maximum one on directed evolutionary forces. It is thought that the following suggestion, while definitely a speculation, offers a possible solution along these lines.

In essence, the proposed hypothesis states that the evolution of the basic syntheses proceeded in a stepwise manner, involving one mutation at a time, but that the order of attainment of individual steps has been in the reverse direction from that in which the synthesis proceeds—i.e., the last step in the chain was the first to be acquired in the course of evolution, the penultimate step next, and so on. This process requires for its operation a special kind of chemical environment; namely, one in which end-products and potential intermediates are available. Postponing for the moment the question of how such an environment originated, consider the

operation of the proposed mechanism. The species is at the outset assumed to be heterotrophic for an essential organic molecule, A. It obtains the substance from an environment which contains, in addition to A, the substances B and C, capable of reacting in the presence of a catalyst (enzyme) to give a molecule of A. As a result of biological activity, the amount of available A is depleted to a point where it limits the further growth of the species. At this point, a marked selective advantage will be enjoyed by mutants which are able to carry out the reaction $B + C = A$. As the external supplies of A are further reduced, the mutant strain will gain a still greater selective advantage, until it eventually displaces the parent strain from the population. In the A-free environment a back mutation to the original stock will be lethal, so we have at the same time a theory of lethal genes. The majority of biochemical mutations in *Neurospora* are lethals of this type.

In time, B may become limiting for the species, necessitating its synthesis from other substances, D and E; the population will then shift to one characterized by the genotype ($D + E = B$, B + $C = A$). Given a sufficiently complex environment and a proportionately variable germ plasm, long reaction chains can be built up in this way. In the event that B and C become limiting more or less simultaneously, another possibility is opened. Under these circumstances symbiotic associations of the type ($F + G \neq C$, $D + E = B$)($F + G = C$, $D + E \neq B$) will have adaptive value.

This model is thus seen to have potentialities for the rapid evolution of long chain syntheses in response to changes in the environment. As has been pointed out by Oparin[11] the hypothesis of a complex chemical environment is a necessary corollary of the concept of the origin of life through chemical means. The essential point of the argument is that it is inconceivable that a self-reproducing unit of the order of complexity of a nucleoprotein could have originated by the chance combination of inorganic molecules. Rather, a period of evolution of organic substances of ever-increasing degree of complexity must have intervened before such an event became a practical, as distinguished from a mathematical, probability. Or, put in another way, any random process which can have produced a nucleoprotein must at the same time have led to the production of a profusion of simpler structures. Oparin has considered in some detail the possible modes of origin of organic compounds from inorganic material and cites a number of known reactions of this type, together with evidences of their large-scale occurrence on the earth in past geologic ages. He concludes that in the absence of living organisms to destroy them highly complex organic systems can have developed. The first self-duplicating nucleoprotein originated as a step in this process of chemical evolution. The origin of living matter by physicochemical means thus

presupposes the existence of a highly complex chemical environment.

To summarize, the hypothesis presented here suggests that the first living entity was a completely heterotropic unit, reproducing itself at the expense of prefabricated organic molecules in its environment. A depletion of the environment resulted until a point was reached where the supply of specific substrates limited further multiplication. By a process of mutation a means was eventually discovered for utilizing other available substances. With this event the evolution of biosyntheses began. The conditions necessary for the operation of the mechanism ceased to exist with the ultimate destruction of the organic environment. Further evolution was probably based on the chance combination of genes, resulting to a large extent in the development of short reaction chains utilizing substances whose synthesis had been previously acquired.

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*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. V. STRAIN RESISTANCE OF BACTERIA TO ANTIBIOTIC SUBSTANCES, ESPECIALLY TO STREPTOMYCIN**

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Different strains of the same species of bacteria are found to vary greatly in their sensitivity to a given antibiotic substance. This phenomenon has an important bearing upon the utilization of the substance for chemotherapeutic purposes, where a knowledge of the sensitivity of the particular strain of a given organism responsible for a certain disease becomes of paramount importance.

It has been definitely established that when a culture of an organism is grown in the presence of a certain antibiotic substance, it becomes resistant to increasing concentrations of the substance; strains may thus be obtained that have become adapted to the action of the antibiotic agent and that require far greater concentrations for growth inhibition as compared with the parent or mother culture from which they were isolated. This has been shown to hold true for the action of lysozyme on *Micrococcus lysodeikticus*, and for various antibiotic and chemical agents against different bacteria. This phenomenon has been studied extensively in recent years as applied to the effect of penicillin on a number of bacteria, including *Staphylococcus aureus*,[1] pneumococci,[10] staphylococci, streptococci,[9] gonococci[2] and various others. Different strains of *S. aureus* have been shown[11] to vary in their susceptibility to penicillin in a range of less than 0.1 unit to greater than 1.0 unit.

Davies, Hinshelwood and Price[3] explained the phenomenon of adaptation of an organism to an antibacterial agent by one or more of the following mechanisms: (1) Adaptation occurs by natural selection from an initially heterogeneous population; this concept has lost, however, much support, since variations have been found to occur in strains derived initially from a single cell. (2) Adaptation occurs by actual modification of the individual cells; this may be due to the establishment in the cells of a mechanism alternative to that normally in use, or a quantitative modification of the existing mechanism occurs. (3) Adaptation is a change in some center of organization of the cell. In summarizing the various theories explaining the phenomenon of bacterial adaptation, Hinshelwood[7] concluded that when variations or adaptive changes occur, there is an actual modification of the character of the individual cells, although selection may be superimposed on this when modified and unmodified cells exist together. Demerec[4] concluded that resistance of *S. aureus* to penicillin originates through a mutation mechanism, and that penicillin acts as a selective agent to eliminate the non-resistant members of the bacterial population.

The adaptation of a certain bacterial strain to a given antibiotic substance does not necessarily signify that the strain is also resistant to another substance. A certain organism may become more resistant to penicillin by growth in gradually increasing concentrations of this antibiotic in an artificial culture medium, or more resistant strains of this organism may be isolated from patients treated with penicillin. Such strains do not necessarily show the same range of resistance to another antibiotic substance, like streptomycin. It was found,[15] for example, in a study of the variation of spore-forming bacteria, that different strains resistant to streptothricin are not at all resistant to clavacin or to fumigacin. Different strains of staphylococci were found[11] to show different

degrees of resistance to penicillin, tyrothricin and streptothricin, the action of one antibiotic not being necessarily paralleled by the action of another. When resistant strains are grown in media free from the antibiotic, a reversion may occur either gradually or suddenly and spontaneously; however, some of the resistant variants may be very stable.[7] The sensitivity of different strains of the same organism to a single antibiotic may also depend upon the morphological and physiological characteristics of the strains, as shown for the sensitivity of sporulating and non-sporulating strains of *Actinomyces griseus* to streptomycin.[14]

In the following investigations a study has been made of the sensitivity and adaptation of different strains of various gram-negative and gram-positive bacteria to streptomycin.[12] This antibiotic agent was selected because of its specific capacity to inhibit the growth of bacteria belonging to both gram-negative and gram-positive groups,[6, 8, 13] and because of its chemotherapeutic potentialities.[8]

TABLE 1

EFFECT OF STREPTOMYCIN UPON DIFFERENT STRAINS OF *E. coli* AND *E. communior*

E. coli		*E. communior*	
CULTURE NO.	D. U.[a]	CULTURE NO.	D. U.
ATCC 26	30	ATCC 130	30
ATCC 6522	30	ATCC 4163	30
ATCC 6880	10	ATCC 4351	30
ATCC 6881	30	ATCC 4352	30
ATCC 8677	100	ATCC 7011	30
ATCC 8739	30	Average	30
W 2	30		
W 4	30		
W 5	30		
Average	35		

[a] D. U. = dilution units per 1 mg. as determined by plate method.

Experimental.—Most of the cultures of bacteria used in these studies were obtained from the American Type Culture Collection (ATCC), though some were taken from our own collection (W), and some were received from special sources, as will be indicated. The streptomycin used in most of the experiments was a dry, fairly stable preparation produced in our own laboratory; it had an activity of 58 units per milligram.

In the first group of experiments, a number of pure cultures of bacteria comprising three gram-negative and two gram-positive species were tested for their sensitivity to streptomycin. The results presented in table 1 show that out of nine strains of *Escherichia coli* and five strains of *E. communior*, only one was very sensitive (100 units) to the antibiotic agent and one was very resistant (10 units); the remaining twelve strains were about equally sensitive, giving about 30 units as measured by the agar streak method.

Greater variation was obtained in the sensitivity to streptomycin of different strains of *Proteus vulgaris* and *S. aureus* tested by the same method, although the average values for these organisms as well were about the same as for *E. coli* (table 2). Out of seven cultures of *Pr. vulgaris*

TABLE 2

EFFECT OF STREPTOMYCIN UPON *S. aureus* AND *Pr. vulgaris*

Pr. vulgaris		*S. aureus*	
CULTURE NO.	D. U.	CULTURE NO.	D. U.
ATCC 6898[a]	30	ATCC 152	25
ATCC 7829	75	ATCC 6518	25
ATCC 8259	50	ATCC 6538	45
ATCC 8427[a]	10	ATCC 8094	30
ATCC 9484	30	ATCC 8431	20
W 1[a]	30	W 1	30
W 2[a]	20	W 2	25
Average	35	W 4	75
		Merck Institute	30
		Average	34

[a] W 1 is same strain as 6898; W 2 is same as 8427.

used in these tests, one was very resistant (10 units) and one was rather sensitive (75 units), the remaining five cultures showing sensitivity to the particular streptomycin preparation ranging from 20 to 50 units, with an average of 35 units. The range in sensitivity of the nine *S. aureus* strains was from 20 to 75 units, with an average of 34 units.

The sensitivity of six strains of *Bacillus subtilis* to streptomycin is brought out in table 3. In the case of this organism, as well, there was considerable

TABLE 3

EFFECT OF STREPTOMYCIN UPON DIFFERENT STRAINS OF *B. subtilis*

	DILUTION (PLATE) METHOD	DIFFUSION (CUP) METHOD. ZONE OF INHIBITION, MM.	
CULTURE	D. U.	DILUTION 1:5	DILUTION 1:25
ATCC 102	30	19.5	13.0
N. R. Smith 237	100	19.0	14.5
N. R. Smith 972	30	16.8	12.0
ATCC 6633[a]	250	26.3	20.5
ATCC 8473	100	23.0	17.5
W 9[a]	250	26.0	20.0
Average	109		

[a] ATCC 6633 is the same strain as W 9.

variation among the different strains. The tests with this organism were carried out by two methods, namely, the agar streak or plate dilution and the agar diffusion or cup methods. The degree of sensitivity of *B. subtilis* to streptomycin was found to be characteristic of the strain rather than of

the organism. Two strains, 102 and 972, were markedly resistant to streptomycin, as measured by both methods, the degree of resistance being of about the same order of magnitude as for the average strain of *E. coli*. Most of the strains of *B. subtilis* were, however, much more sensitive. The most sensitive strain was available in two different cultures, namely, ATCC 6633 and W 9; both showed the same degree of sensitivity by both methods of testing. Strain 237 produced by the cup method, in addition to the clear, easily readable zones, also secondary and even tertiary zones, which tended to confuse the readings, since only the clear zones were measured. Finally, strain 8473 did not produce the same type of growth as did the other strains of *B. subtilis;* it formed rough surface colonies which gave the plates a peculiar appearance, although they did not interfere with the reading of the zone of inhibition. With the exception, therefore, of the last two cultures, the strains of *B. subtilis* varied in their sensitivity to streptomycin as much as 10:1.

TABLE 4

BACTERICIDAL EFFECT OF STREPTOMYCIN UPON *Pr. vulgaris*

INCUBATION WITH STREPTOMYCIN, HRS.	STREPTOMYCIN PER 1 ML. CULTURE			
	0	2	10	50
	MILLIONS OF CELLS PER 1 ML.			
0	1,290	1,290	1,290	1,290
6	2,150	1,010	665	100
24	1,470	110	30	2.3
48	745	1.2	0[a]	0[a]
124	360	0	0	0

[a] 1–4 colonies on a plate from 1:100,000 dilution.

In order to establish the manner in which strains of a given culture are capable of developing resistance against streptomycin, *Pr. vulgaris* was selected for more detailed studies. At first, the effect of the concentration of streptomycin upon its bactericidal action was determined by using a single strain (No. 2) of *Pr. vulgaris*. The results of a typical experiment are given in table 4. When 2.0 to 10 units of streptomycin were added to a culture of *Pr. vulgaris* all the cells were killed in 48 to 124 hours; when one unit was used, there was only a temporary inhibition of growth, which was later overcome. Cultures of *Pr. vulgaris*, treated with the larger amounts of streptomycin, were streaked, after 6 days' incubation, on nutrient agar plates, and the colonies produced from a few surviving cells were isolated and transferred to fresh media. A new strain was thus obtained possessing all the cultural and staining properties of the mother culture; however, it proved to be more resistant to streptomycin. This new strain was designated as R. Two original strains and the resistant

form were inoculated into several lots of fresh broth containing varying concentrations of streptomycin. The numbers of viable bacteria were determined after several periods of incubation at 37°C. The results reported in table 5 show emphatically that strain R, isolated from culture No. 2, became highly resistant to the action of streptomycin.

The sensitivity of the original culture of *Pr. vulgaris* and that of the resistant strain R was now measured against streptothricin, an antibiotic substance closely related to streptomycin. The results, which need not be reported here in detail, tended to demonstrate that strain R, made resistant to streptomycin, also became, though perhaps not to so great a degree, resistant to streptothricin, thus pointing to a certain similarity in the two substances. When, however, a totally different type of antibiotic agent was used, namely, clavacin, no difference could be found in the sensitivity of the streptomycin-resistant strain R as compared to that of

TABLE 5

BACTERICIDAL ACTION OF STREPTOMYCIN UPON *Pr. vulgaris* AND A FRESHLY ISOLATED RESISTANT STRAIN OF THIS ORGANISM

Number of Cells in Millions per 1 Ml.

STREPTOMYCIN UNITS PER 1 ML.	CULTURE W 1	CULTURE W 2	STRAIN R
	AT START		
	39.5	45.5	26.0
	AFTER 28 HRS.' INCUBATION		
0	375.0	615.0	560.0
1	0.01	0.65	190.0
5	0[a]	0[a,b]	9.0
25	0	0[a,b]	3.13

[a] 1 colony on a plate from 1:1000 dilution.
[b] 7–8 colonies on a plate from 1:10 dilution.

the mother culture No. 2. Thus, a strain of a bacterium that becomes resistant to a given antibiotic substance may also be resistant to a related compound, but not to a different type of antibiotic substance.

The results of a more detailed study of this phenomenon, using the mother culture and the resistant strain R of *Pr. vulgaris*, as well as two corresponding strains of *S. aureus*, are presented in table 6. With the first organism, the previous observations are confirmed. With *S. aureus*, however, the strain made more resistant to streptomycin was not rendered resistant to streptothricin. A possible explanation for this discrepancy may be found in the fact that the resistance of *S. aureus* to streptomycin was not increased sufficiently. The ratio of the activity of streptomycin against the parent strain of *Pr. vulgaris* to that against the resistant strain is about 12:1, whereas the ratio of the activities of streptothricin against these two strains is 6:1. With *S. aureus*, however, streptomycin was

only five times less active against the resistant strain than against the parent strain. Thus, if one assumes that these two agents act similarly against *Pr. vulgaris* and *S. aureus*, the resistance to streptomycin of the new strain of *S. aureus* is not sufficiently higher than that of the parent strain for differences in their resistances to streptothricin to become apparent.

The action of several antibiotic substances upon four cultures of *S. aureus* and their corresponding strains made resistant to streptomycin was now determined. These cultures were obtained from The Merck Institute of Therapeutic Research. The ratio of sensitivity to streptomycin of the resistant strains to that of the mother cultures varied from 750 to >2000. When tested against streptothricin, the corresponding ratios were 1 to 4, thus indicating only a very low degree of increase in resistance. When tested against clavacin and St, an antibiotic substance

TABLE 6

BACTERIOSTATIC ACTIVITY OF ANTIBIOTIC SUBSTANCES UPON STREPTOMYCIN-RESISTANT STRAINS OF TWO BACTERIA

	ACTIVITY, DILUTION UNITS PER MG.[a]				
	Pr. vulgaris			*S. aureus*	
ANTIBIOTIC AGENT	STRAIN 1	STRAIN 2[b]	RESISTANT STRAIN R	PARENT STRAIN	RESISTANT STRAIN
Streptomycin	250	100	8	250	50
Streptothricin	250	150	25	250	250
Clavacin	150	150	150	200	250

[a] The activity of the antibiotic agents was for streptomycin, 58 units; streptothricin, 500 units; and for the crystalline clavacin, 200 units; the actual results obtained for streptomycin were multiplied by 8.3, namely, the activity ratio of the streptothricin and streptomycin preparations, in order to make them comparable.

[b] Strain 2 is the parent culture from which the resistant strain R has been isolated.

produced by an aerobic spore-forming bacterium, no difference whatsoever in the sensitivity of the original cultures and streptomycin-sensitive strains could be detected.

Summary.—Different strains of the same bacterial species may vary greatly in their sensitivity to streptomycin. The ratio of sensitivity to a given preparation varied for *E. coli* from 100 to 10 units with an average of 35; for *Pr vulgaris*, 75 to 10 units (average 35); for *S. aureus*, 20 to 75 units (average 34); and for *B. subtilis*, 30 to 250 units (average 109).

A streptomycin-resistant strain of *Pr. vulgaris* showed also a certain degree of resistance to streptothricin but none at all to clavacin.

A strain of *S. aureus* that was made only slightly resistant to streptomycin showed no resistance to streptothricin. Several highly resistant strains of *S. aureus* showed no increase in resistance to clavacin and to an antibiotic substance isolated from a spore-forming soil bacterium, and only a trace of increased resistance to streptothricin.

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

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*STABILIZING INFLUENCE OF LIBERAL INTAKE OF VITAMIN A**

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Recently we have reported evidence that liberality of nutritional intake of vitamin A tends to postpone aging and to increase the length of life.[1]

It is now found further that offspring of the (rat) families having the higher vitamin A intakes grow not only somewhat more rapidly but also with distinctly lesser individual variability, thus indicating both a favorable and a stabilizing influence, of liberal vitamin A, upon the growth process.

Table 1 shows the mean weights at specified ages and the mean gain in weight from the 28th to the 56th days of age (end of infancy to early adolescence in the rat) for strictly parallel series of experimental animals on Diets 16, 360 and 361, which are otherwise alike but contain, respectively, 3, 6 and 12 International Units of vitamin A per gram of air-dry food; or 0.8, 1.6 and 3.2 I. U. per food calorie.

It will be seen that early growth is more rapid on the diets with 6 or 12 than on that with 3 I. U. per gram; while the growth was essentially alike on the two higher levels. This was equally true for each sex. Weights

TABLE 1

INFLUENCE OF THREE LEVELS OF DIETARY INTAKE OF VITAMIN A UPON GROWTH OF RATS: BODY WEIGHTS AT FIXED AGES, AND GAINS IN 5TH TO 8TH WEEKS OF AGE, IN GRAMS; WITH C. V. OF GAINS. (THE NUMBERS OF CASES ARE GIVEN IN PARENTHESES)

	MALES			FEMALES		
	ON DIET WITH 3 I. U./G.	ON DIET WITH 6 I. U./G.	ON DIET WITH 12 I. U./G.	ON DIET WITH 3 I. U./G.	ON DIET WITH 6 I. U./G.	ON DIET WITH 12 I. U./G.
Mean weight of offspring, continued on respective family diet, at age of						
28 days	(94) 43	(84) 44	(81) 45	(92) 43	(91) 42	(75) 43
56 days	(93) 98	(84) 114	(78) 115	(92) 87	(91) 101	(75) 102
84 days	(73) 160	(61) 184	(59) 184	(90) 129	(79) 151	(64) 149
100 days	(67) 189	(56) 215	(53) 213	(78) 146	(72) 166	(55) 163
120 days	(67) 215	(56) 244	(52) 242	(82) 163	(77) 173	(61) 172
150 days	(67) 250	(56) 273	(52) 271	(72) 177	(71) 187	(55) 184
180 days	(59) 273	(48) 293	(46) 290	(65) 186	(63) 193	(48) 190
210 days	(59) 288	(48) 308	(43) 304	(65) 193	(61) 203	(49) 199
240 days	(57) 300	(48) 319	(43) 314	(65) 199	(63) 206	(48) 205
270 days	(57) 308	(48) 325	(43) 321	(65) 203	(63) 215	(50) 212
300 days	(57) 313	(48) 328	(43) 326	(63) 207	(61) 217	(47) 217
365 days	(37) 320	(36) 334	(28) 334	(47) 219	(43) 231	(38) 229
400 days	(35) 322	(36) 334	(28) 336	(48) 221	(40) 231	(32) 233
500 days	(30) 323	(33) 327	(22) 323	(46) 229	(42) 236	(33) 235
600 days	(26) 309	(30) 308	(17) 306	(40) 220	(41) 230	(33) 231
700 days	(18) 291	(22) 294	(13) 292	(39) 213	(38) 222	(29) 223
730 days	(14) 296	(18) 292	(13) 287	(37) 212	(34) 220	(27) 221
Gains in weight, 28th to 56th days of age:						
Means in grams	(93) 54.2±0.89	(84) 69.6±0.81	(78) 70.4±0.70	(92) 44.0±0.90	(91) 59.2±0.53	(75) 59.2±0.55
Coefficient of variation	28.3	15.9	13.1	29.3	12.8	12.2

of pregnant females were not included in the averages for their respective age groups. Adult weights will be seen to be essentially alike for the 6- and 12-unit levels, and relatively little higher than for the corresponding animals on the intake-level of 3 units per gram. The coefficients of variation of the body weights, as distinguished from gains, were small in all cases.

Much more striking are the facts brought out in the lower section of table 1 which show that in the period of rapid growth, between the 28th and 56th days of the life of these rats, the coefficient of variation of the individual data of the respective groups or series are, in both sexes, much larger for those on the 3 I. U. than for those on the 6- and 12-I. U. levels of vitamin A per gram of food. Yet rat families in our colony are thriving in the 58th generation on the diet containing even the lowest of these three levels.

We conclude that while 3 I. U. of vitamin A per gram of air-dry food (or 0.8 I. U. per food calorie) fully meets the requirements of adequacy, as the word is commonly understood, there is a somewhat higher and a much less variable rate of growth when the level of vitamin A intake is twice or four times higher.

This stabilizing effect appears to be a further advantageous influence of the same liberal levels of dietary vitamin A that have previously been shown[1] beneficial to adult vitality and length of life.

* Aided by grants from The Nutrition Foundation, Inc.

[1] Sherman, H. C., Campbell, H. L., Udiljak, M., and Yarmolinsky, H., these PROCEEDINGS, **31**, 107–109 (1945).

ILLUSTRATIONS AND SIMPLE ABSTRACT PROOF OF SYLOW'S THEOREM

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Sylow's theorem in the theory of finite groups consists of the following three parts: If the order of a group G is divisible by p^m, p being a prime number, but not by a higher power of p, then G contains at least one subgroup of order p^m, and if it contains more than one such subgroup all of these subgroups are conjugate under G and their number is of the form $1 + kp$. The first of these three parts can be proved independently of the other two parts. In fact, these two parts follow almost directly from the first of these three parts. Hence this part will receive most of our attention in what follows.

A Norwegian mathematician, L. Sylow (1832–1918), was the first to publish this general theorem. It appeared in the French language in a German mathematical journal called *Mathematische Annalen*, **5**, 584–594 (1872). This was about two years after the first treatise on group theory was published by a well-known French mathematician, C. Jordan (1838–1922). The title of this very influential work is *Traité des substitutions et des équations algèbriques* (1870). Various parts of this standard work of XVIII + 667 large pages could have been much simplified by the use of Sylow's theorem if it had then been known. Important steps toward its proof had been taken earlier. In particular, the noted French mathematician, A. L. Cauchy (1789–1857), published a proof of the fact that if the order of a group is divisible by a prime number then the group contains a subgroup whose order is equal to this number and hence the theorem has often been called the Cauchy-Sylow theorem. This special case had been stated without proof by E. Galois (1811–1832).

When G is an abelian group it can be proved very easily that Sylow's theorem applies to it by using the following obvious theorem relating to the order of the product of two commutative group operators. This order is the product of all the powers of the prime numbers which appear to a higher power in the order of one of these two operators then in the order of the other and the product of divisors of powers of prime numbers appearing to the same highest powers in the orders of the two given operators. The same theorem may be expressed by saying that if p^α is the highest power of p which divides the order of one of the two given operators but is not a divisor of the order of the other then it is a divisor of the order of their product but if p^α is the highest power of p which divides the orders of both of these operators and neither of them is divisible by a higher power of p then the order of their product is divisible by no higher power of p than p^α. The product of all such powers of prime numbers is the order of the product of tl e two given group operators.

From this theorem it follows directly that if none of the operators of the abelian group G has an order which is divisible by p then the order of G cannot be divisible by p and if the order of such an operator of G is divisible by p this operator is the product of an operator whose order is a power of p and an operator, which may be the identity, whose order is prime to p. Hence all the operators of G may be supposed to be so represented that some of them have orders which are powers of p while the rest of them have orders which are prime to p. The former will therefore generate a subgroup whose order is a power of p while the latter generate a subgroup whose order is prime to p and G is the direct product of these two subgroups. The order of the former subgroup is the highest power of p which divides the order of G and this is therefore the Sylow subgroup of order p^m contained in G.

It remains to consider the case when G is non-abelian, and it may first be noted that it may be assumed that G is one of the non-abelian groups of smallest order to which Sylow's theorem does not apply in case it does not apply to G. If G contains more than one invariant operator all of its invariant operators generate an invariant subgroup of G. Since both the order of this subgroup and the order of the corresponding quotient group are smaller than that of G it follows that Sylow's theorem applies to both of them and hence it also applies to G, which is contrary to the stated hypothesis. It therefore follows that it may be assumed that the identity is the only invariant operator of G.

All the operators of G can be arranged in sets of conjugates such that no two sets have any operator in common and that the identity alone constitutes one of these sets. Each of the operators of one of the sets is transformed into itself by all the operators of a subgroup of G and the number of the operators in the set is equal to the order of G divided by the order of a subgroup of G. Hence there results the following equation:

$$g = 1 + g_1 + g_2 + \ldots + g_n.$$

In this equation g represents the order of G and $g_1, g_2, \ldots, g_n$ represent the numbers of the operators in the given sets of conjugates. It should be noted that each of the numbers $g_1, g_2, \ldots, g_n$ usually exceeds unity and is equal to g divided by the order of a subgroup of G.

Since p divides g it cannot divide each of the numbers $g_1, g_2, \ldots, g_n$. Hence there is at least one of these numbers which is prime to p and each of the corresponding subgroups must have a common order which is divisible by p^m. These subgroups are found in G and hence G contains a subgroup of order p^m. To prove that all the subgroups of order p^m in G form a single set of conjugates it may be noted that a given one of these subgroups could not transform into itself another one of them since g is not divisible by a higher power of p than p^m. Therefore it results that if there were more than one set of such subgroups it would follow that if those of a set were transformed by one of their conjugates the number of the subgroups in the set would be of the form $1 + kp$ and if those of the same set were transformed by one of those in another set of conjugates this number would have to be of the form kp. Since this is a contradiction it has been proved that all these subgroups of order p^m are conjugate under G and that their number is of the form $1 + kp$.

The equation

$$g = 1 + g_1 + g_2 + \ldots + g_n$$

is fundamental. When $n = 1$, G is obviously the group of order 2. When

$n = 2$ G is either the group of order 3 or the symmetric group of order 6. When G is any abelian group n is clearly equal to $g - 1$ and hence it is only necessary to consider the cases when G is non-abelian. In all of these cases n is less than $g - 1$ and at least equal to the number of the distinct prime numbers which divide g. If it is equal to this number g cannot be divisible by the square of a prime number and hence it contains then an invariant subgroup whose order is the largest prime number which divides g. Hence the symmetric group of order 6 is the only group in which n is no larger than the number of the distinct prime numbers which divide g when G is a non-abelian group.

For every prime divisor p of g there is at least one of the numbers $g_1, g_2, \ldots, g_n$ which is prime to p and the sum of all such numbers when this sum is increased by unity is divisible by p. This includes the well-known theorem that every group of order p^m contains at least $p - 1$ invariant operators besides the identity, since the number of the operators in every set of conjugates of such a group is divisible by p whenever the set contains more than one operator. Hence the formula $g = 1 + g_1 + g_2 + \ldots + g_n$ which is useful in proving Sylow's theorem is also useful in the study of the prime power groups. A necessary and sufficient condition that G is an abelian group is that each of the numbers $g_1, g_2, \ldots, g_n$ is unity and hence n has then its maximal value.

When G is non-abelian the maximal value of n will clearly result when the number of the invariant operators of G is as large as possible and each of the non-invariant operators of G has only two conjugates under G provided that both of these conditions can be satisfied at the same time. This can be done in the present case. In fact, the largest number of the invariant operators of a non-abelian group can clearly not exceed the order of the group divided by 4 and when it is equal to this number the commutator subgroup of the group is of order 2 and hence each of the non-invariant operators of G has exactly two conjugates under G. In other words, when G is a non-abelian group the maximal value of n is $5g/8 - 1$ and the central of G is of order $g/4$ while its commutator subgroup is of order 2. The octic group is clearly the smallest group which satisfies these conditions.

A necessary and sufficient condition that the non-abelian group G contains a maximal number of sets of conjugate operators is that it contains three abelian subgroups of index 2. If it contains two such subgroups it also contains three such subgroups and its central is of index 4 under G while its commutator subgroup is of order 2. The theorem that a non-abelian group has three, one, or no abelian subgroups of index 2 is a special case of the theorem that a non-abelian group of order p^m has $p + 1$, one, or no abelian subgroup of index p, where p is a prime number. If a non-abelian group of order p^n contains more than one abelian subgroup of

index p the number of its sets of conjugate operators is $p^{m-2} + p^{m-1} - p^{m-3}$ and hence the maximal value of n in this case is $p^{m-3}(p^2 + p - 1) - 1$. This evidently reduces to $5g/8 - 1$ when $p = 2$ as was noted above.

ON THE NUMBER OF SOLUTIONS OF CERTAIN NON-HOMOGENEOUS TRINOMIAL EQUATIONS IN A FINITE FIELD

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The relation

$$au^m + bv^m + 1 = 0, \tag{1}$$

where a and b are given elements of a finite field F, with u and v to be determined in F, with $abuv \neq 0$, has been studied by Mitchell.[1] For the special case where F consists of residue classes with respect to a prime modulus, this equation has been studied by a number of writers.[2] All the methods used by these writers appear to depend on a certain symmetry arising from the fact that the exponents of u and v are the same.

However, in another paper[3] the writer obtained, based mainly on extensions of a method due to V. A. Lebesgue,[4] an expression for the number of roots of equation (1) modulo p which was independent of this symmetry. Hence, we shall apply this latter method to the consideration of

$$au^e + bv^f + w^g = 0 \tag{1a}$$

and

$$au^e + bv^f + 1 = 0. \tag{1b}$$

We shall find expressions for the number of solutions (u^e, v^f, w^g) of equation (1a) and the number of solutions (u^e, v^f) of equation (1b) in a finite field F, providing that $abuvw \neq 0$. If we obtain this number, then we may immediately find the number of solutions (u, v, w). Also we may confine ourselves to the case where e, f and g are divisors of $p^n - 1$, where the order of F is p^n, when p is prime, as the other cases depend on this.

Using Theorem II of my previous paper,[3] we determine N in the relation

$$N = \sum(1 - (ax_1 + bx_2 + x_3)^{p^n-1}), \tag{2}$$

when the summation extends over x_1, x_2, x_3, where x_1 ranges independently over all the distinct values such that $x_1^h = 1$, similarly x_2 over all roots of $x_2^i = 1$, and x_3 over all roots of $x_3^j = 1$, where

$$\frac{p^n-1}{e}=h; \qquad \frac{p^n-1}{f}=i; \qquad \frac{p^n-1}{g}=j.$$

To reduce equation (2) we write

$$(ax_1+bx_2+x_3)^{p^n-1}=\sum_{k_1=0}^{p^n-1}(ax_1+bx_2)^{k_1}(x_3)^{p^n-1-k_1}\binom{p^n-1}{k_1}. \qquad (2a)$$

Now

$$\binom{p^n-1}{k_1}\equiv(-1)^{k_1}(\text{mod } p); \qquad (2b)$$

and we may write, by further expansion,

$$(ax_1+bx_2+x_3)^{p^n-1}=\sum_{k_1=0}^{p^n-1}\sum_{r_1=0}^{k_1}(ax_1)^{r_1}(bx_2)^{k_1-r_1}(x_3)^{p^n-1-k_1}(-1)^{k_1}\binom{k_1}{r_1}$$

in the field. The summation of this with respect to x_3 leaves only terms involving $\binom{jk}{r_1}$, since $p^n-1-k_1\equiv 0(\text{mod } j)$ must hold for such terms, and we set $jk=k_1$. Similarly, summation with respect to x_1 gives $hr=r_1$, and with respect to x_2 gives $jk-hr\equiv 0(\text{mod } i)$. This yields

$$\sum_{x_1,\,x_2,\,x_3}(ax_1+bx_2+x_3)^{p^n-1}=hij\sum_{k=0}^{g}\sum_{r}\binom{kj}{hr}a^{hr}b^{kj-hr}(-1)^{kj},$$

where r ranges over the values d in the set such that $jk-hd\equiv 0(\text{mod } i)$. Using this relation with equation (2), we have

THEOREM I. *If N is the number of distinct solutions (u^e, v^f, w^g) of the equation*

$$au^e+bv^f+w^g=0$$

in a finite field F of order p^n, which contains a, b and c; $abuvw\neq 0$, then

$$N=-hij\sum_{k=1}^{g}\sum_{r}\binom{kj}{hr}a^{hr}b^{kj-hr}(-1)^{kj} \qquad (3)$$

in F. Here e, f and g each divide p^n-1;

$$h=\frac{p^n-1}{e}; \qquad i=\frac{p^n-1}{f}; \qquad j=\frac{p^n-1}{g};$$

and r ranges over the values d in the set

$$0, 1 \ldots \left[\frac{jk}{h}\right]$$

such that $jk-hd\equiv 0$ (mod i).

In the same way we may consider the equation

$$au^e + bv^f + 1 = 0, abuv \neq 0, \tag{4}$$

and obtain by the same method, for the number of its solutions (u^e, v^f) in $F(p^n)$

$$-hi \sum_{k=1}^{f} \sum_{r=0}^{c} \binom{ki}{hr} a^{hr} b^{p^n-1-ki} (-1)^{ki}, \tag{5}$$

where

$$c = \left[\frac{ki}{h}\right].$$

We may then state the following:

THEOREM II. *If N is the number of distinct sets of solutions (u^e, v^f) in a finite field $F(p^n)$ of*

$$au^e + bv^f + 1 = 0$$

where $abuv \neq 0$, then N has the form (5), in the field.

The number of solutions of equation (4) is at most $p - 1$, for $n = 1$, so that we have the

THEOREM III. *The number of sets of distinct integral solutions (u^e, v^f) of*

$$au^e + bv^f + 1 \equiv 0 (\text{mod } p), \tag{6}$$

where a and b are integers, p is prime $(abuv, p) = 1$,

$$h = \frac{p-1}{e}, \qquad i = \frac{p-1}{f},$$

is the least residue $\geqq 0$ of

$$-hi \sum_{k=1}^{f} \sum_{r=0}^{c} \binom{ki}{hr} a^{hr} b^{p-1-ki} (-1)^{ki} \tag{7}$$

modulo p, where

$$c = \left[\frac{ki}{h}\right].$$

In the proof of Theorem I we could also have written the right-hand member of equation (2a) in the form

$$\sum_{k_1=0}^{p^n-1} (ax_1 + x_3)^{k_1} bx_2^{p^n-1-k_1} \binom{p^n-1}{k_1},$$

as well as

$$\sum_{k_1=0}^{p^n-1} (bx_2 + x_3)^{k_1} ax_1^{p^n-1-k_1} \binom{p^n-1}{k_1}.$$

This shows that if we call the expression (7), $A(h, i, j)$, then this equals in the field, each one of the expressions obtained by interchanging the letters h, i, j in the proof of Theorem I, namely: $A(i, h, j)$, $A(i, j, h)$, $A(j, i, h)$, $A(h, j, i)$ and $A(j, h, i)$; and these are all equal to N in the field. This fact does not appear easy to prove by any direct method.

As an application, the number, N, of sets of distinct integral solutions (x^4, y^2) of

$$ax^4 + by^2 + 1 \equiv 0 (\text{mod } p)$$

where a and b are integers, p is prime $(axby, p) = 1$, and $h = \frac{p-1}{4}$, is seen by Theorem III to be equal to the least integral residue modulo $p \geqq 0$ of

$$-\frac{p-1}{4} \cdot \frac{p-1}{2} \sum_{k=1}^{2} \sum_{r=0}^{2k} \binom{2kh}{hr} a^{hr} b^{p-1-2kh} (-1)^{2kh}.$$

Modulo p, this reduces, after using relation (2b), to

$$-1/8 \left(b^{2h} + \binom{2h}{h} a^h b^{2h} + a^{2h} b^{2h} + 1 + (-1)^h a^h + a^{2h} + (-1)^h a^{3h} + 1\right). \tag{8}$$

As is known p can be expressed uniquely in the form

$$p = (|r|)^2 + (|s|)^2 \tag{8a}$$

with r and s of different parity. Let us select s to be positive and even and choose the sign of r so that r has the form $4n - 1$ if p has the form $8n + 1$, but so that r has the form $4n + 1$ if p is of the form $8n + 5$. It is also known[5] that in this case

$$(-1)^{h+1} 2r \equiv \binom{2h}{h} (\text{mod } p). \tag{8b}$$

Let g be a primitive root of p defined by the congruence

$$rg^h + s \equiv 0 (\text{mod } p). \tag{8c}$$

Such a g will always exist; and a can be expressed as g^t;

$$t = 0, 1, 2, 3.$$

Therefore, using relations (8b) and (8c), we find

$$N \equiv -1/8 (b^{2h} + (-1)^{h+1} 2rg^{th} b^{2h} + g^{2th} b^{2h} + 5), \tag{9}$$

if

$$(-1)^h g^{th} \equiv 1 (\text{mod } p); \tag{10}$$

and

$$N \equiv -1/8(b^{2h} + (-1)^{h+1} 2rg^{th}b^{2h} + g^{2th}b^{2h} + 1), \tag{11}$$

if

$$(-1)^h g^{th} \not\equiv 1 (\text{mod } p). \tag{12}$$

Let either $t = 0$, and h be even; or $t = 2$, and h be odd. Then relations (9) and (10) apply, and congruence (9) reduces to

$$N_1 \equiv -1/8 \left(2\left(\frac{b}{p}\right) - 2r\left(\frac{b}{p}\right) + 5\right), \tag{13}$$

where $\left(\frac{b}{p}\right)$ is the Legendre symbol. Now let either $t = 0$, and h be odd; or $t = 2$, and h be even. Then congruences (11) and (12) apply, and relation (11) reduces to

$$N_2 \equiv -1/8 \left(2\left(\frac{b}{p}\right) + 2r\left(\frac{b}{p}\right) + 1\right), \tag{14}$$

while if $t = 1$ or $t = 3$, then relation (11) applies and can be written, using relation (8c)

$$N_3 \equiv -1/8 \left((-1)^h 2s\left(\frac{g}{p}\right)^{(t-1)/2}\left(\frac{b}{p}\right) + 1\right). \tag{15}$$

These formulae determine N as a least positive or zero residue modulo p. However, it is possible when $p > 13$ to exhibit N as an integer without any reduction with respect to the modulus p, as shall now be shown.

Designate by α_d, $d = 1, 2, 3$, respectively, the number $-8N$ in relations (13), (14) and (15). Then

$$|\alpha_1| \leqq 7 + 2|r|, |\alpha_2| \leqq 3 + 2|r|, |\alpha_3| \leqq 1 + 2s$$

and from relation (8a)

$$|r| < \sqrt{p}, s < \sqrt{p}$$

so

$$|\alpha_d| \leqq 7 + 2\sqrt{p}.$$

When $p > 13$, then it is easy to show that

$$7 + 2\sqrt{p} < p, \tag{15a}$$

and

$$|-\alpha_d| + 7p < 8p. \tag{16}$$

Now since $N \equiv -1/8\alpha_d (\text{mod } p)$, or $8N \equiv -\alpha_d + zp$, it follows that $0 \equiv$

$-\alpha_d + zp \pmod 8$. Since p and 8 are relatively prime, this linear congruence in z has just one non-negative integral solution $\theta < 8$.

$$8N \equiv -\alpha_d + \theta p \pmod p. \tag{17}$$

Since $|-\alpha_d + \theta p| < 8p$, and N is the least non-negative integral solution of equation (17), therefore N equals the lesser non-negative one of the following two numbers:

$$-1/8(\alpha_d - \theta p); \qquad -1/8(\alpha_d - (8 + \theta)p).$$

The same reasoning will apply to the case $p = 13$ if $\alpha_d \not\equiv 0 \pmod{13}$.

THEOREM IV. *The number, N, of sets of distinct integral solutions* (x^4, y^2) *of*

$$ax^4 + by^2 + 1 \equiv 0 \pmod p \tag{18}$$

where a and b are integers, p is a prime > 13 *of the form* $4h + 1$, *and* $(axby, p) = 1$, *is equal to the lesser non-negative one of the following two numbers:*

$$-1/8(\alpha_d - \theta p),$$
$$-1/8(\alpha_d - (8 + \theta)p),$$

where θ *is the least non-negative integral solution* z *of the congruence*

$$0 \equiv -\alpha_d + zp \pmod 8,$$

and where α_d, $d = 1, 2, 3$, *is taken to be equal, respectively, to* $-8N_1$, $-8N_2$, $-8N_3$ *in congruences (13), (14), (15).*

COROLLARY. *The congruence (18) of Theorem IV for* $p > 13$ *always has solutions.*

This follows from the inequality (15a) and the fact that α_d is odd and hence $\neq 0$.

[1] *Ann. Math.*, II, **18**, 120–131 (1917).

[2] Cf. the references given by Mitchell in his article above cited, as well as Dickson, *Am. Jour. Math.*, **57**, 391, 463 (1935); *Trans. Amer. Math. Soc.*, **37**, 363 (1935).

[3] These PROCEEDINGS, **30**, 362–367 (1944).

[4] *Jour. de Math.*, I, **2**, 260–266 (1837). The writer was in error in ascribing this method, as applied to trinomial congruences involving rational integers, to Hurwitz. Lebesgue in the reference just cited went much further than Hurwitz, obtaining a result which is closely related to Theorem III of the article referred to in the third footnote above, page 367.

[5] Bachmann, *Die Lehre von der Kreisteilung*, 133, 135, 136 (1872).